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Wnt/β-Catenin As Anticancer Drug Target

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ABSTRACT

Wnt /β-catenin signaling plays an important role in tumor cell dedifferentiation and proliferation. Wnt proteins are a family of secreted glycoproteins. Abnormal activation of wnt signaling results in tumor progression. Wnt proteins binds to the frizzled receptors and LRP5/6 co-receptors and through the stabilization of β-catenin a critical mediator, initiates a complex signaling cascade that plays an important role in regulating cell proliferation and differentiations. The elevated levels of oncogenic protein-β-catenin have been observed in many of the human cancers, indicating that this pathway plays an important role in tumor development. The wnt signaling can be inhibited at the extracellular level, at regulatory protein level and also by targeting the expressions of β-catenin by protein knockdown and by targeting the downstream mediators of β-catenin signaling such as c-myc, cyclin D1, PPARS and COX-2. In this review we discuss the effects of NSAIDS and flavanoids that are being used or explored to target the β-catenin signaling in the treatment of cancers.

Keywords: wnt, β-catenin, signal transduction, anticancer targets, NSAIDS, flavanoids

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INTRODUCTION

Cancer is viewed as a multi-stage disease caused by the accumulation of genetic alterations in tumor suppressor genes or oncogenes. Activation of the wnt/β-catenin signaling pathways is important in human tumorigenesis. Many component of this signaling pathway may serve as rational targets of cancer drug target development.

The term "wnt" was coined from a combination of the Drosophila segment polarity gene *Wingless* and the mouse proto ontogeny *Int-1*[1]. The Wnt signaling pathway plays a key role in tumor cell de differentiation and proliferation. Evidence suggests that abnormal activation of Wnt pathway plays a vital role in tumor progression. In general, tumor formation occurs due to the abnormal Wnt signaling by nuclear accumulation of β-catenin as a result of glycogen synthetase kinase- 3 (GSK-3) inactivation. Wnt signaling becomes deregulated through multiple mechanisms leading to cancer; particularly colorectal cancer, for which adenomatous polyposis coli (APC) or β-catenin are mutated in approximately 95% of tumors.[2] Wnt is comprised of a diverse family of secreted lipid-modified signaling glycoproteins that are 350–400 amino acids in length. The lipid modification that occurs on these proteins is palmitoylation of cysteines. Palmitoylation is necessary as it initiates targeting of the Wnt protein to the plasma membrane for secretion and it allows the Wnt protein to bind its receptor due to the covalent attachment of fatty acids. Wnt proteins also undergo glycosylation, which attaches a carbohydrate in order to insure proper secretion. In Wnt signaling, these proteins act as ligands to activate the different Wnt pathways via paracrine and autocrine routes.[3]

The Pathway

Wnt signaling begins when a Wnt protein binds to the N-terminal extra-cellular cysteine rich domain of a Frizzled (Fz) family receptor[4]. These receptors span the plasma membrane seven times and constitute a distinct family of G-protein coupled receptors (GPCRs)[5]. However, to facilitate Wnt signaling, co-receptors may be required such as lipoprotein receptor-related protein(LRP)-5/6, receptor tyrosine kinase (RTK), and ROR2[3]. Upon activation of the receptor, a signal is sent to the phosphoprotein Dishevelled (Dsh), which is located in the cytoplasm. This signal is transmitted via a direct interaction between Fz and Dsh. Dsh proteins are present in all organisms and they all share the conserved protein domains: an amino-terminal DIX domain, a central PDZ domain, and a carboxy-terminal DEP domain. These different domains are important because after Dsh, the Wnt signal can branch off into multiple pathways and each pathway interacts with a different combination of the three domains[6]

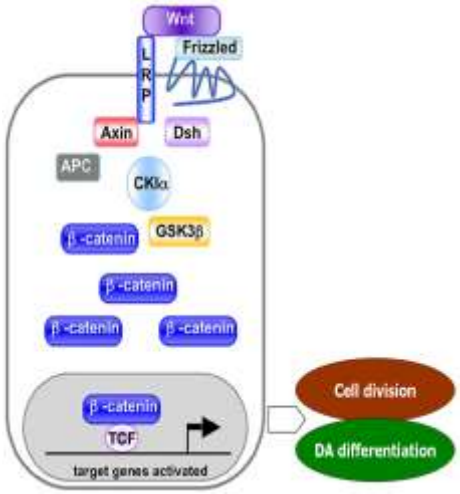

Figure 1: Schematic representation of Wnt/β-catenin signaling

Canonical pathway

The canonical Wnt pathway (or Wnt/_-catenin pathway) leads to regulation of gene transcription. it causes an accumulation of β-catenin in the cytoplasm and its eventual translocation into the nucleus to act as a transcriptional coactivator of transcription factors that belong to the TCF/LEF family. Without Wnt signaling, β-catenin would not accumulate in the cytoplasm since a destruction complex would normally degrade it.[6]

Noncanonical pathways

The noncanonical planar cell polarity (PCP) pathway regulates cytoskeleton responsible for shape of the cell. It does not involve β-catenin and LRP-5/6 as its co-receptor and is thought to use NRH1,Ryk, PTK7 or ROR2. The PCP pathway is activated via the binding of Wnt to Fz and its co-receptor. The receptor then recruits Dsh, which uses its PDZ and DIX domains to form a complex with Dishevelled-associated activator of morphogenesis 1 (DAAM1). Daam1 then activates the small G-protein Rho through a guanine exchange factor. Rho activates Rho associated kinase(ROCK), which is one of the major regulators of the cytoskeleton. Dsh also forms a complex with rac1 and mediates profilin binding to actin. Rac1 activatesJNK and can also lead to actin polymerization. Profilin binding to actin can result in restructuring of the cytoskeleton and gastrulation.[7]

Noncanonical wnt/calcium pathway

The noncanonical Wnt/calcium pathway also does not involve _-catenin. Its role is to help regulate calcium release from the endoplasmic reticulum (ER) in order to control intracellular calcium levels. the activated Fz receptor directly interacts with Dsh and activates specific Dsh-protein domains. The domains involved in Wnt/calcium signaling are the PDZ and DEP domains. The Fz

receptor directly interfaces with a trimeric G-protein. This co-stimulation of Dsh and the G-protein can lead to the activation of either PLC or cGMP-specific PDE. If PLC is activated, the plasma membrane component PIP2 is cleaved into DAG and IP3. When IP3 binds its receptor on the ER, calcium is released. Increased concentrations of calcium and DAG can activateCdc42 through PKC. Increased calcium also

activates calcineurin and CaMKII. CaMKII induces activation of the transcription factor NFAT, which regulates cell adhesion, migration and tissue separation. Calcineurin activates TAK1 and NLK kinase, which can interfere with TCF/ß-Catenin signaling in the canonical Wnt pathway.[8] However, if PDE is activated, calcium release from the ER is inhibited.

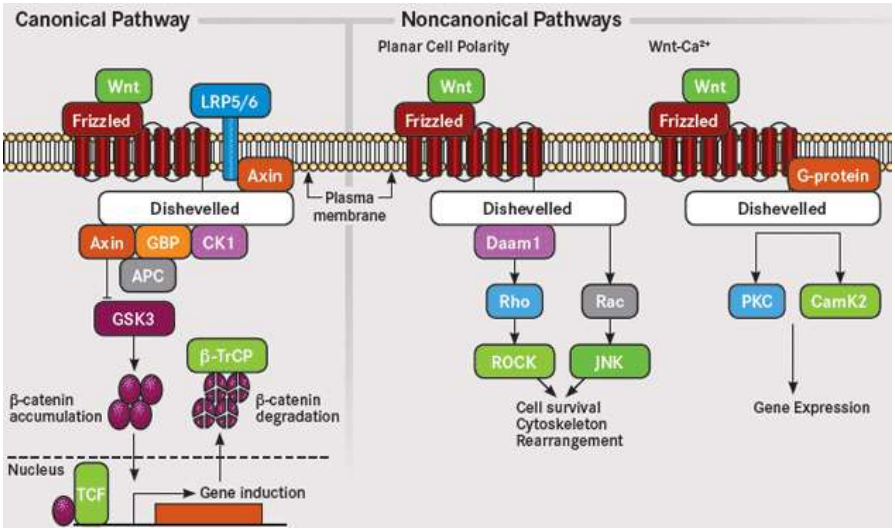

Figure 2: Schematic representation of wnt pathways

INHIBITION OF WNT/B-CATENIN SIGNALING

The pathway can be inhibited by targeting at various levels such as:

Targeting wnt/βcatenin signaling at the extracellular level

Targeting growth factors and or their receptors have recently generated a great deal of interest especially with the recent success of trastuzumab which is a monoclonal antibody that selectively binds to the extracellular domain of HER2 . It is conceivable that the wnt ligands themselves can be targeted for anticancer therapy.[9]

Targeting wnt/βcatenin signaling at the regulatory protein level

Wnt/βcatenin signaling is subjected to tight regulation exerted by a network of intracellular protein-protein interactions. Many of the proteins are negative regulators, which functions as tumor suppressors in this signaling pathway. The beta catenin binding domain of APC is sufficient for tumor suppression, mini-APC fragment may be used as anticancer agent. Similarly Axin another negative regulator of this pathway is considered as a tumor suppressor because Axin is

mutated in several types of human cancers. Several other negative regulators of wnt beta catenin signaling can also be conceptually developed as anticancer drugs. These factors include Idax, Axam and ICAT.[10]

Targeting downstream mediators of β catenin signaling

There are many genes regulated by beta catenin/Tcf, some of the genes that have been associated with human cancers includes c-Myc, cyclin D1,PPARδ,COX-2, CD44, MMP7 and c-Myb. It is possible that these genes can also be targeted for efficacious cancer therapy as many of these downstream targets may play an important role in tumoregenesis,[11]

c-Myc:

c-Myc is an oncogene over expressed in a variety of human cancers including melanoma, leukaemia and prostate and colon carcinomas. Upregulated by wnt, c-Myc seems to be a viable target for anticancer therapy. Antisense oligonucleotides have been shown to have anti-proliferative effects in leukaemia, lymphoma, melanoma, prostate, breast and liver cancer cells.[12]

Cyclin D1

Cyclin D1 expression is trans activated by the beta catenin /Tcf complex. Cyclin D1 complexes with CDK4 (cyclin dependant kinase 4) and CDK6 to phosphorylate retinoblastoma(Rb) which promote histone acetylation and gene transcription. Cell cycle progression is tightly regulated by the cyclin dependant kinases and the cyclins.[13]

PPARs and COX-2

PPARs and or COX-2 may be regulated by wnt /beta catenin signaling pathway. COX-2 may also be regulated directly or indirectly by PPARs. COX-2 has been implicated in many human cancers such COX inhibitors such as NSAIDs can effectively inhibit the development of colon cancers. PPARδ is a downstream target of wnt signaling. The PPARs are nuclear receptors that bind to lipophillic ligands and the receptor for 9-cis-retinoid(RXR) to trans activate genes. There are three PPAR genes(PPARα,PPARβ/δ and PPAR γ) with two PPARγ isoforms isoforms PPARγ has been implicated in many human cancers and studies have shown anti neoplastic effects of PPARγ agonist in neuroblastoma, astrocytoma, glioma and prostate, thyroid, colon, pancreatic, hepatocellular, breast and lung carcinomas[14]

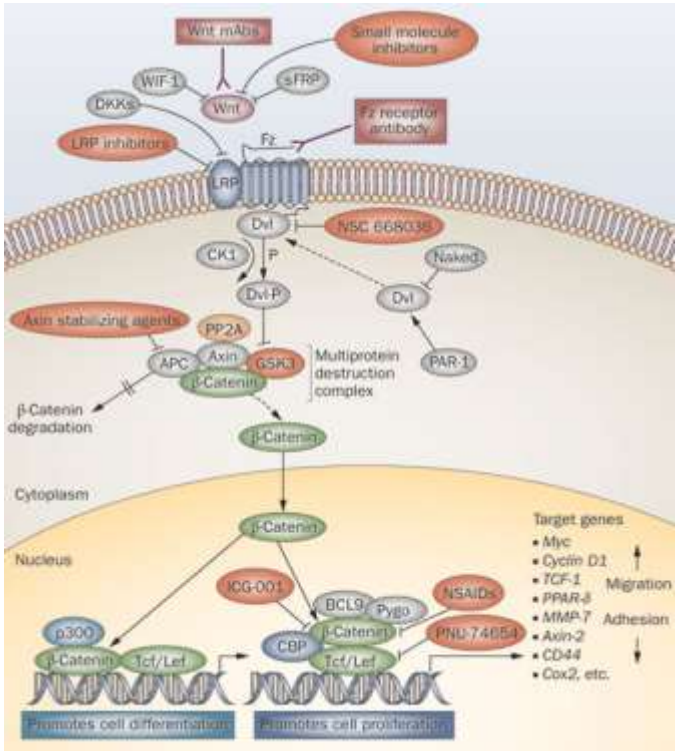

Figure 3 schematic representation of inhibition of Wnt signaling

DRUGS INHIBITING THE WNT PATHWAY IN ANTICANCER THERAPY

NSAIDS

Celecoxib:

Celecoxib, a cyclooxygenase-2 (COX-2) selective nonsteroidal anti-inflammatory drug, Its antitumor effects depend on the one hand on its COX-2-inhibiting potency, but on the other hand on COX-2-independent mechanisms. celecoxib has an impact on the APC/β-catenin pathway, which has been shown to play a pivotal role in the development of various cancers, especially of the colon.[15]Beta-catenin expression is regulated by the APC protein. As a consequence of APC-mutation, nearly all colon carcinoma tissues show significant over expression of β-catenin, which has been attributed to be crucial for the early stages of colorectal carcinogenesis [15]. Phosphorylation of β-catenin by the GSK-3β kinase is required for its ubiquitination and degradation by the ubiquitin-proteasome pathway [16]

Inhibition of the GSK-3β by various stimuli leads to dissociation of the APC/axin/β-catenin complex and cytosolic β-catenin accumulation . Free unphosphorylated β-catenin translocate into the nucleus. This nuclear translocation is regulated by several transcription factors of the TCF (T cell-factor) family such as TCF-4 and lef-1 (lymphoidenhancer-factor-1), which were shown to interact with β-catenin. The β catenin/TCF complex recruits further chromatin-remodeling proteins to responsive promoters, thereby activating the transcription of specific target genes, including c-

myc, c-jun, fra-1, cyclin D1 and cyclooxygenase-2 (COX-2)[17]. Cyclooxygenase-2 (COX-2), which is the key enzyme in the conversion of arachidonic acid to prostaglandins. Because prostaglandins were shown to promote cell proliferation, angiogenesis and metastasis, and to inhibit the induction of apoptosis [18], the anticarcinogenic effects of

NSAIDs were primarily attributed to their COX-2 inhibitory activity. However, recent studies have suggested also COX-2-independent mechanisms to explain the anticarcinogenic effects of NSAIDs. For example, it has been shown that celecoxib has an antitumorigenic effect in COX-2-deficient tumors and induces apoptosis in cells which do not express COX-2

SAR:

Selective COX-2 inhibitors possess 1,2 diaryl substitution on a central hetero or carbocyclic ring system with methane sulphonyl, sulfonamide, azido, methane sulphanamide or pharmacophore based tetrazole group on one of the aryl ring that plays a crucial role on COX-2 selectivity.COX-2 inhibitory potency and selectivity is dependent on the nature of substituents on the C2 phenyl ring,[19]The order of selectivity OH>F>OHe>H, Me>NHCOMe>Cl Substitution of OH group at para position of C2 phenyl ring yields most potent COX-2 inhibitors. An increase in the size of nitrogen substituent improved the potency,[20]

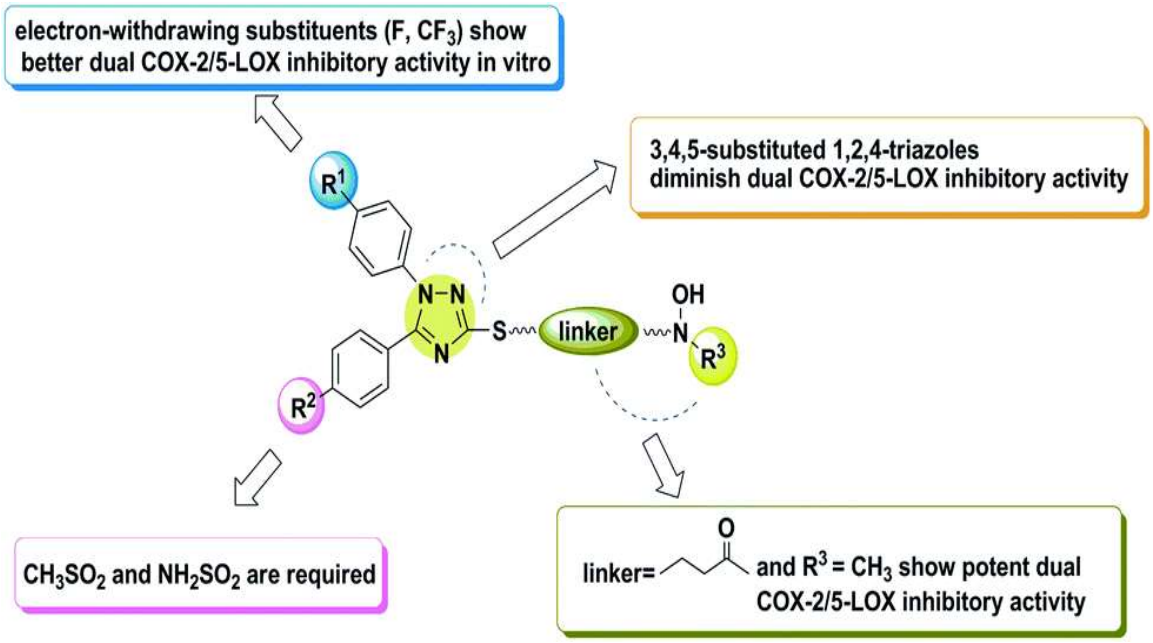

Figure 4: schematic representation of SAR of celecoxib

Ibuprofen:

Nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the cyclooxygenase (COX)-1 and COX-2 enzymes, have an impressive history as effective chemopreventive agents for colorectal cancer. These effects are largely dependent on their ability to inhibit the production of proliferative

and inflammatory prostaglandins (PG), most notably prostaglandin E2 . More recently, specific COX-2 inhibitors were developed as a means to reduce the gastrointestinal toxicity associated with inhibiting COX-2. Apart from their abilities to inhibit COX activity and prostaglandin production, both selective and nonselective COX-2 inhibitors can target b-catenin, a key mediator of colon tumorigenesis. It can increase the phosphorylation of b-catenin, thus decreasing its nuclear accumulation and transcription of Wnt/b-catenin target genes, such as cyclin D1, c-myc, and PPARd[21-23]. It was found that ibuprofen treatment inhibited the accumulation of nuclear b-catenin expression, an outcome that correlated with reduced levels of cyclin D1.

SAR:

It posses a centre of acidity, which can be represented as carboxylic acid, an enol, a hydroxamic acid, a sulphanamide or a tetrazole. The centre of acidity is generally located one carbon atom adjacent to a flat surface represented by an aromatic or hetero aromatic ring. The distance between these centre is critical because increasing this distance to two or three carbons generally diminishes activity. Substitution of a methyl group on the carbon atom separating the aromatic ring tends to increase anti inflammatory activity. In ibuprofen, acetic acid group is essential for activity. Benzene ring may be substituted with other aromatic ring. Aryl side chain can be replaced with other hydrophobic groups or halogens such as fluorine or chlorine.[24]

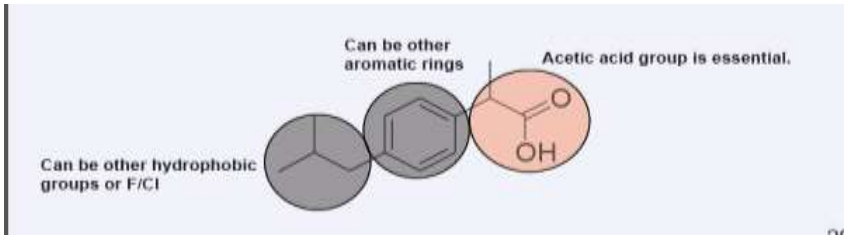

Figure 5 schematic representation of SAR of Ibuprofen[32]

FLAVONOIDS:

Flavonoids are modulators of several cellular signaling pathways such as the Wnt/β-catenin pathway (canonical Wnt signaling) [25]. These molecules act on different components of the Wnt signaling pathway, such as Dkk1 , Gsk3-β , Dsh and β- catenin/TCF/LEF[26-28]. The flavonoid blocks the Wnt signaling, most likely at the nuclear complex, between β-catenin and TCF ,and inhibits the phosphorylation of β catenin S33A.[28]

QUERCETIN:

Quercetin is a negative modulator of the Wnt/β-catenin signaling pathway. Quercetin shows high and non-specific toxicity, affects the proliferation of glioblastoma cells, with lower toxicity. These

anti-proliferative effects were accompanied by changes in β-catenin cellular localization, suggesting that Wnt/β-catenin signaling might be altered by this flavonoid.[28]

SAR:

Hydroxyl groups and planar conformation of the Quercetin has an important role for antioxidant activity. Methylation of hydroxyl groups or incorporation of bulky groups like rutinosyl and hydroxyethyl. O-methylation of all hydroxyl groups resulted in complete loss of antioxidant activity whereas O-methylation at 5 and 7 position has less effect on antioxidant activity. The compound with rutinosyl group at 3-O position has less antioxidant capacity than the Quercetin and 3-O methylated compound[28].

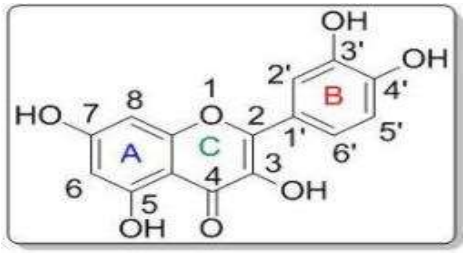

Figure .6 schematic representation of SAR of Quercetin

GENISTEIN:

It is an isoflavone derivative. Genistein treatment of cancer cells upregulates GSK-3β expression and enhances GSK-3β association with β-catenin, leading to β-catenin phosphorylation and degradation. As consequence Genistein inhibits prostate cancer. Genistein also suppressed β-catenin/Tcf trancriptional activity in SW480 cells (colorectal carcinoma cells) . Genistein affect the upstream components of the β-catenin/Tcfpathway by suppression of GSK-3β and Akt phosphorylation. Genistein also modulatesWnt pathway in other cell types. Genistein reduces cell proliferation through the recruitment of β-catenin to membrane and reducing cytosolic β-catenin. In addition, Genistein reduces the protein and mRNA levels of cyclin D1.[29]

SAR:

Substitution by hydroxyl group enhances the activity whereas substitution by methoxy group diminishes the activity of isoflavonoids. The position of substitution appears to be a determining factor for the activity. Hydroxyl group substitution was of utmost importance at the C4 position, of moderate importance at C5 position and of little importance at C7 position .Loss of 2,3 double bond coupled with absence of 4-oxo group confers greatest activity. Methylation or oxygenation on the phenyl ring does not increase cytotoxic activity against cancer cells. Stability and toxicity against cancer cells can be improved by substitution of acetylated sugar hydroxyls, a C=C in sugar

molecule binding directly to aglycone, localization of sugar substituent atC7-OH position in genistein molecule.[29]

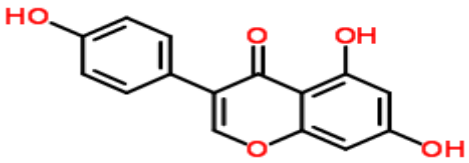

Figure 7 schematic representation of SAR of Genistein

CONCLUSION

Cancer can be therefore perceived as a disease of communication between and within the cells. The role of signaling pathways in cancer is as pleiotropic as cancer itself. Inhibitors of the signaling pathways provides effective antineoplastic agents for the treatment of a wide range of human cancers, including those where current regimen of drugs are disappointingly ineffective. Gain or loss of fuction mutations of several members of wnt catenin pathway, such as β-catenin, Axin or APC, have been found in many cancers. The identification of these regulatory proteins offered the development of new therapies targeting this pathway at the extracellular/membrane, cytoplasmic and nuclear levels and a synergistic approach of targeting wnt-dependant and independent β-catenin activating pathway. At the cytoplasmic level, potential drugs like axin, APC based viral and DNA vectors , tyrosine kinase inhibitors or COX-2 inhibitors have been developed. It can be proposed that flavanoids inhibit carcinogenesis as these are potent antioxidants. Reactive oxygen species are supposed to be involved in DNA damage, cell division, cell signaling and growth. It is also speculated that flavanoids can inhibit angiogenesis, hence, flavanoids may seem to play an important role in anticancer therapy.

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